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Outline For

OFFICE OF NAVAL RESEARCH

Microbiology Branch

SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: John W. Brown, M. D. Date: July 16, 1952
C. V. Seastone, M. D. For Period: Jan. 1 to June 30, 1952

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CONTRACTOR: The University of Wisconsin
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TITLE OF PROJECT: ACUTE RESPIRATORY INFECTIONS

Objectives: 1. Study of acute beta hemolytic streptococcic infections, including the pathogenesis of rheumatic fever. An attempt to relate antigenic and enzymatic properties of apparently infecting strains of beta hemolytic streptococci to clinical and antibody responses of individual patients is an important aspect.

2. Study of acute respiratory diseases of unknown or obscure etiology, particularly the search for etiologic agents hitherto unknown.

The essential principle is based upon the belief that the GENERAL APPROACH to this difficult field must take advantage of every promising development, and probably will require major changes in direction. To this end, the search for methods and for CONCEPTS, different from those already explored by competent investigators, seems important. Application of new methods for isolation of hitherto unknown agents and the use of more effective serologic or other means is an aspect. The diagnosis of several common infections (influenza, Herpes, etc.) is essential, but incidental, during the search for other entities. Repetition or duplication of methods, or study of diseases already under appropriate scrutiny, are not the purpose of this project. Correlation of the knowledge available to the investigators, who represent several related fields, is considered important. At any time, circumstances may dictate a change in approach not now apparent. All aspects of the studies in progress have as the major ultimate objective, an approach to the solution of the problems of prevention and control (including treatment) of acute respiratory infections of known or unknown etiology.

Abstract (or Summary) of Results.

a. and b. Since start of project to the present (since date of activation was October 1, 1951):

1. The current work on beta hemolytic streptococcal infections, in collaboration between the Naval Medical Research Unit No. 4 at the U. S. Naval Training Center, Great Lakes, Illinois, and the University of Wisconsin, began during the winter of 1950. During the current report period, the studies have been particularly related to an attempt to evaluate the antigenic and enzymatic properties of apparently infecting strains of beta hemolytic streptococci with the clinical and antibody response of individual patients. There are 111 individuals and strains of beta hemolytic streptococci which caused or were associated with infection in them upon which the data considered necessary for evaluation is at hand. A great many more individual patients were seen and studied clinically, and many additional strains of beta hemolytic streptococci isolated and studied, but on which the necessary critical information was not available, as dictated by the rigid criteria for this study. The 111 form the basis for the evaluation and results. Of these, 60 were naval recruits and 51, students at the University of Wisconsin.

The relationship between the ability of the strain to produce streptokinase, hyaluronidase, hyaluronic acid and the antibody response to streptolysin O, streptokinase, and hyaluronidase in the individuals studied was evaluated. Failure to show a rise in antibody to a given streptococcal product was observed, although the infecting organism elaborated that product. Antibody increases were frequently observed when the organism isolated could not be shown to give rise to the corresponding antigen. There was no consistent relationship between the Lancefield type and the formation of streptokinase, hyaluronic acid or hyaluronidase. Antibiotic therapy appeared to have a consistent but not marked influence in inhibiting antibody response to streptolysin O, streptokinase, and hyaluronidase.

Four patients acquired rheumatic fever. Three had received penicillin therapy within the first, second, and third days, respectively, after onset of symptoms of pharyngitis. The antibody response of these 4 patients was exceptionally high, but not higher than others who recovered uneventfully. Three of the strains produced hyaluronic acid alone; one produced both hyaluronic acid and hyaluronidase; two produced streptokinase; and two did not.

A separate study to determine the antibody production and tuberculin sensitivity in individuals with a history of rheumatic fever is pertinent to this problem. The approach was based on the concept that individuals who contract rheumatic fever have reacted in a hypersensitive manner to a stimulus provided by the beta hemolytic streptococcus. It was thought conceivable that rheumatic individuals might also react in an abnormal manner to any antigenic stimulus. The precipitating antibody response to Type 1 pneumococcal polysaccharide was not significantly different in a group of 19 individuals with rheumatic heart disease or a history of rheumatic fever, and a group of 22 individuals without rheumatic heart disease or a history of rheumatic fever. Sensitivity to tuberculin following BCG immunization was not significantly different in 12 rheumatic individuals and 20 non-rheumatic subjects. Detectable opsonins were demonstrated consistently in sera containing more than 0.5 mg. of pneumococcal antibody per 4.0 ml. of serum.

Although there is considerable information as to antigenic and enzymatic properties of various strains of beta hemolytic streptococci and of the antibody response of groups of individual patients to several antigenic constituents of beta hemolytic streptococci, there is little information available to date with which to evaluate the antigenic properties of an apparently infecting hemolytic streptococcus with the clinical and antibody response of the individual patient from which it was isolated during acute pharyngitis. The studies reported here suggest that there is a greatly increased need for further clinical investigation along those lines and that current concepts relative to the pathogenesis of rheumatic fever must remain unconfirmed pending further studies.

2. a. Studies on the role of Coxsackie viruses in respiratory disease:

Preliminary studies were undertaken early in July, 1951. Attempts were made to recover Coxsackie virus from stool specimens, collected at the U. S. Naval Training Center, from patients with acute febrile diseases with respiratory symptoms. Fifteen individuals were studied. Coxsackie virus was recovered from one.

Each member of a training company of 72 healthy recruits were surveyed by means of rectal swabs on entrance into training. None of these revealed a C virus. The same procedure was done at the end of the training period (October, 1951) with similar results. A few of the isolation experiments were performed at NAMRU-4, by inoculation of suckling mice supplied from the University of Wisconsin.

During the interval, 1 of 3 stool specimens obtained from children in Madison (suspected of having poliomyelitis or Coxsackie virus infection) was found to contain Coxsackie virus.

Specific complement fixing antigens and antisera (stable) have been prepared in the laboratory at the University of Wisconsin to those which are at present standard for Coxsackie viruses.

b. The use of newly developed tissue culture technics. Attempts to isolate specific etiologic agents so far unknown.

Dr. Lois K. Kitze spent part of January, 1952, in the laboratory of Dr. John Enders and his associates for the purpose of familiarizing herself with his methods. Subsequently, technical proficiency with these procedures has been developing, and the use of the method is currently being employed , using frozen nasal washings of 6 individuals among a group of 46 patients with pneumonia who were seen by the staff of NAMRU-4. The results are not yet available. The methods are being extended. It is anticipated that several acute respiratory infections of unknown otiology will be studied in this way. It is not likely that many specimens from any group of patients can receive the time-consuming efforts necessary. The rewards, by way of contribution, which are potentially possible by isolation of one hitherto unknown agent would justify all of the effort involved.

c. Clinical and laboratory survey of 46 consecutive patients with pneumonia observed at NAMRU-4 between November 7, 1951, and March 25, 1952:

The patients were unselected and consecutive in the sense that every one appearing in a certain sequence (under a planned treatment alternation schedule) is included. The 46 patients had close observation, serial chest x-rays, blood counts, sputum cultures (when available) nose and throat cultures. Nasal washings from each patient, with few exceptions, were studied for the presence of influenza virus. Blood serum samples were obtained at appropriate intervals and studied for antistreptolysin O and Hirst titer. These studies were performed at NAMRU-4.

A sample of sputum, nasal washings, blood clot, and acute and convalescent serum (each in a frozen state) were transported to the University of Wisconsin. These are on file in both laboratories. The sera have been examined for cold agglutinins and heterophile antibodies. Thirteen of the patients' sera were studied for antibodies to Q fever and the Psittacosis-lymphogranuloma group. The frozen nasal washings are the subject of special isolation technics mentioned above. The attempt to recover Newcastle disease virus of fowls and vesicular stomatitis virus of cattle and horses from nasal washings of 6 of these have been made by the Department of Veterinary Science.

The group of 46 pneumonia patients presents unusual features. These patients may represent several causative agents. The results have been equivocal. The clinical data is superior. The data obtained are on file, and further studies along the lines indicated are contemplated. Better lines of approach should be found.

(1). Psittacosis-lymphogranuloma group complement fixing antibodies in serum:

Complement fixation tests for this group of etiologic agents were done on 13 sera of the group of pneumonia patients. All were negative except 2, which gave very slight positive reactions in the micro-complement fixation plate test with serum dilutions of 1:4 to 1:8. This was not considered significant, but these sera are being retested by the standard tube method.

(2). The same sera, referred to above under 2. c. (1) were tested by complement fixation test for antibodies to Q fever, ~~rickettsea~~. All were negative.

(3). Newcastle disease of fowls and vesicular stomatitis virus of cattle and horses:

Isolation experiments to recover either Newcastle disease virus or vesicular stomatitis virus were performed on 6 among the 13 patients whose sera were tested as noted immediately above. Frozen nasal washings were used. Newcastle disease virus or vesicular stomatitis virus were not isolated in any of the 6.

(4). Cold agglutinins were examined in the sera of all but 5 of the 46 patients. All examined were negative except for 1. In this patient the acute serum revealed a titer of 1:128 and the convalescent serum of 1:64.

(5). Heterophile antibodies:

All sera except that from 5 patients in the group of 46 were examined for heterophile antibodies. Each was negative.

(6). Attempt to isolate agents of unknown nature;

The same 6 patients, whose frozen nasal washings were used in the attempt to recover Newcastle disease virus and vesicular stomatitis virus, are being studied by special technics as indicated above. The studies have not yet been completed.

Each of these various lines of approach constitutes a major problem.

Eight of the 46 patients with pneumonia reported here had a positive nose or throat culture for group A, beta hemolytic streptococci during the course of their illness. These were isolated and grouped and typed at NAMRU-4. At the same time, the carrier rate was quite high among non-infected individuals in the same community.

Influenza virus was isolated from 3 individuals' nasal washings out of the 42 who were examined at NAMRU-4. Pneumococci of a specific type (mostly higher types) were isolated from 7 of the 43 patients whose sputa were examined at NAMRU-4.

The tests noted immediately above were performed at NAMRU-4, as were the ASO and Birst titers on blood serum. Final reports from the collaborating laboratory there must remain pending.

Plans For Future:

Immediate:

1. The study of the relationship of antigenic and enzymatic properties of infecting beta hemolytic streptococci to clinical and antibody responses of patients has suggested several questions. The "recall" effect of minimal amounts of antigen is one of those. This aspect may be related to the effect of penicillin therapy which might be expected to reduce the amount of antigen of any type within an infecting streptococcus and therefore reduce the stimulus provided. The specific anti-H substance of Lancefield is not expected to be involved in this recall mechanism. As noted above, an antibody response did occur to anti-streptolysin O and several other antigenic components of the infecting streptococcus in many of the patients studied.

Research on streptococcal disease will probably be mainly devoted to the question of virulence or invasiveness. This is involved and difficult. It is almost impossible to anticipate the direction of study. The possible interrelation of the M substance in hyaluronic acid is being studied and will undoubtedly continue for some time.

2. A single season's experience with 1 recruit company does not suggest that "C" viruses are of significance in the etiology of respiratory diseases in naval recruits. More study is essential during other seasons with other individuals for any conclusion to be reached in this respect. The plans include repeating the survey. More specimens from persons with symptoms suggesting this type of infection will be studied. As indicated, serologic identification of viruses isolated is possible now. The specific complement fixing antigens and antisera have been prepared in the laboratory at the University of Wisconsin.

At the present time several hundred stool specimens are being collected from recruits at the U. S. Naval Training Center by the personnel of NAMRU-4 in the course of a survey for intestinal parasites. About every fifth stool specimen is to be frozen and preserved for study for Coxsackie viruses as soon as feasible. Another recruit company will be picked up early after arrival at Great Lakes. This company will have the examinations for C viruses which were made last year. It is anticipated that several individuals among the group will be selected with respect to site of origin so as to cross as many geographic lines within the United States as possible. During the latter part of July and August, a few individuals with respiratory infections of various kinds, and those of acute gastrointestinal nature, will have isolation experiments performed, using their stools.

3. Continued isolation experiments with specimens from patients with acute respiratory diseases will be made to evaluate the significance, if any, of vesicular stomatitis, Newcastle disease, Herpes simplex, and influenza. Suitable groups of recruits with respiratory infections and students at the University of Wisconsin with similar diseases will have serologic studies continued for the purpose of surveying the group for Q fever antibody, lymphocytic choriomeningitis, Psittacosis-lymphogranuloma group, and mumps by complement fixation tests.

Long Range:

The long term plans include the accumulation of reliable clinical data and appropriate specimens in cases of acute respiratory diseases at NAMRU-4 and at the University of Wisconsin. This material is intended for use as developments dictate. The long range objectives are essentially those outlined in the original proposal. The principle of approach and objectives outlined therein and at the beginning of this report have not been altered by the experience to date. Results so far have not suggested any major change.

Of most importance to the long range study will be the continued development of technics and their use in the attempt to isolate agents which may be etiologic for acute respiratory infections but which have, to date, not been recognized. If one hitherto unknown agent was isolated in this way, the subsequent epidemiologic therapeutic and subsequently preventive measures which would become a logical sequence, would justify all of the efforts involved in a series of negative experiments. It is anticipated that several acute respiratory infections of unknown etiology will be studied in this way. However, it is not likely that many specimens from any group of patients can receive the time consuming efforts necessary. The investigators have had experience with acute respiratory infections diseases in the clinic, laboratory, and in the field. It is critical to the isolation experiments attempted that those concerned employ the utmost good judgment in the selection of cases and of specimens from them for isolation experiments.

Studies suggested include plans for epidemiologic surveys. When animal diseases are concerned, a veterinary scientist and an epidemiologist of the group will join. As long term aspects for consideration, the syndrome of infectious mononucleosis, infectious hepatitis, and leptospirosis will be considered.

Central nervous system involvement during acute infections is a problem of considerable interest. An electroencephalograph apparatus is being used at the University of Wisconsin Infirmary Hospital to obtain tracings on as many cases of acute respiratory infections as possible. Many abnormal tracings have been obtained in patients with "non-bacterial pneumonia," infectious mononucleosis, mumps, and herpetic stomatitis. Most of these individuals did not give clinical evidence that involvement of the central nervous system was a feature. This study will require detailed evaluation and a great many more observations.

ADMINISTRATIVE:

Dr. A. F. Rasmussen, formerly Professor of Medical Microbiology and Preventive Medicine, University of Wisconsin, who participated in a most critical manner in developing and continuing the studies reported, has taken the position of Professor and Chairman of the Department of Medical Microbiology at the University of California at Los Angeles. Dr. Duard Walker, a virologist and clinical investigator of demonstrated initiative and proven ability, will take his place. It is anticipated that Dr. Walker will have an equal interest and carry on with the studies reported in a manner similar to that of Dr. Rasmussen.

Dr. R. W. Quinn, formerly Associate Professor of Preventive Medicine, University of Wisconsin Medical School, has left to become Professor and Chairman of the Department of Preventive Medicine at Vanderbilt University School of Medicine, Nashville, Tennessee. Dr. Alfred S. Evans, also a clinical investigator of proven ability, now Chief of the Hepatitis Research Center of the U. S. Army in the European Command will soon return to take the place of Dr. Quinn. The arrangements in personnel at the University of Wisconsin will probably provide unaltered continuation or the approach to the project described. Developments and time have increased the effectiveness of the technical aspects.

REPORTS AND PUBLICATIONS:

None